Title of the thesis

Isolation and identification of electrogenic bacterial strain from wastewater of rice based industries for bioelectricity generation using Microbial Fuel Cell technology

Introduction

Starting from the origin of the universe, energy is the base for its existence. Earth is monitored by the power of sun, the sole source of energy to maintain the life on it. Energy which is expressed in terms of joule (J) is the backbone of life which is the capacity to perform work. Forms of energy include mechanical energy (potential energy + kinetic energy), heat energy, chemical energy, electrical energy, light energy, nuclear energy and bioenergy *etc*. Universal law of energy conservation states that it is neither be produced nor be destroyed it only changes its form from one to another. All kinds of energy transformations and situations obey the law of conservation of energy.

Human depend largely on carbon based fossil fuels which are categorized as the non-renewable resources (take thousands of years to be formed). Over exploitation of non-renewable resources consequently gave rise to global warming and other unusual environmental phenomenon. The looming consequences associated with non-renewable resources have shifted the human eyes to focus on renewable resources which can replenish within timeframes to get argent reaps on environmental issues. Re-evaluation of the current energy policies around the world is concerns to use the waste materials as a new source for biofuels and bioenergy production providing exciting sustainable alternative to these problems (Srikanth and Mohan, 2012).

Conventionally, microbes have been used in many of the beneficiary works ranging from common bakery to commercially specialized products. Recently, variety of bioenergy processes and technologies are specifically linked with microbial communities (Wei *et al*., 2011 and Wang and Ren, 2013). Conventional fuel cell devices convert chemical energy to electrical energy directly (Wingard *et al.*, 1982). Advancement of these well practised techniques combined the biological entities to give rise to bioelectrical systems that convert biomass spontaneously into bioenergy like-biohydrogen, biogas, and bioelectricity through the metabolic activity of the living organisms (Berk and Canfield, 1964). More recent studies with microorganisms and enzymes offered modifications of

bioelectrochemical systems namely microbial fuel cell and enzymatic fuel cells as the components of fuel cell (Wingard *et al*., 1982).

Bioelectricity is generation of electricity from the living beings. About a century ago in 1910, Potter introduced the concept of microbial fuel cells (Potter, 1911). This work inspired rise of new interdisciplinary research on the horizon of alternative energy technology towards novel solutions. Microbial fuel cells are devices that employ microbial consortiums, capable of generating electricity known as "eletricigens" or "exoeletricigens" or "anode respiring bacteria" that have ability to oxidize organic compounds to carbon dioxide while directly transferring electrons to electrodes (Lovley, 2006; Lovley and Nevin, 2008). Earlier reports suggest two configurations of MFCs either single chambered (Logan *et al.*, 2008) or dual chambered (Berk and Canfield, 1964).

A typical MFC have three major components; electrodes, separator and electricigens. Previous literature suggests some carbon based, graphite based and metal based electrodes. For example- carbon cloth, carbon paper, carbon felt (Logan and Regan, 2006), graphite based electrodes like graphite fibre brush, graphite felt (Logan *et al.*, 2008), graphite granules, carbon mesh (Wang *et al.*, 2009) and platinum, platinum black, activated carbon (Singh and Songera, 2012) for anode and cathode material. Electrodes can be arranged in single or tubular or multi-electrode configurations (Kim *et al.*, 1999). These electrodes should have properties of being biocompatibility, stability, high electrical conductivity and large surface area (Logan *et al.*, 2008 and Singh and Songera, 2012). Cathode can be exposed to air or other additional electron acceptors like permanganate, chromium hexacynoferate and azo dye *etc.* (Kalathil *et al.*, 2013). The separator, either a cation exchange membrane (Kim *et al.*, 2002) or salt bridge (Mohan *et al.*, 2008) is used to keep cathode and anode chambers separated, and allow proton transport from anode to cathode chamber. The potential difference generated between two chamber drives the electrons to run on the circuit while microbial degradation of waste materials provided as substrate to generate bioelectricity (Pant *et al*., 2011).

Earlier studies describe two distinct type of MFC. First is mediator-less MFC that uses electrochemically active bacteria to transfer electron directly to electrode (Kim *et al.*, 2002). Second is with mediator MFC which employ mediator for the transfer of electrons. Few of the common mediators are thionine, methylene blue, neutral red, *etc*. (Daniel *et al.*, 2009). Earlier reports clear another important mechanism of electron transport that is use

of self-produced mediator by bacteria and a specialized structure called nanowires or pilli of *Geobacter* sp. (Kalathil *et al*., 2013). Microbial communities frequently studied in an MFC includes *Geobacter sulfurreducens* (Caccavo *et al*., 1994), *Shewanella putrefaciens* (Kim *et al*., 2002), *Saccharomyces cerevisiae* (Potter, 1911), *Escherichia coli*, *Proteus vulgaris* (Kim *et al*., 2007), *Rhodoferax ferrireducens* (Chaudhuri and Lovley 2003) and *Geothrix fermentans* (Bond and Lovley, 2005). Performance of MFC depends on multiple factor like substrate concentration that is applied organic load, nature of substrate, substrate degradation rate, biofilm formation on the electrode's surface, external and internal resistance, mode of operation and type of electrodes, *etc.* (Lovley and Nevin, 2008 and Wei *et al.*, 2011).

Applications of MFCs include a variety of advantages like long-term sustainability, use of renewable resources, degradation of organic and inorganic waste, wastewater treatment, biohydrogen production, and removal of compounds like nitrate *etc*. (Lovley and Nevin, 2008). Electrochemically-active microbial community required a better understanding for full-scale implementation and exploitation of its capacities in an MFC and needs aid in efforts to increase electricity generation. This area demands for continue studies. With these concerns utilizing waste may serve as raw material in all consideration. Challenges in this field include wide applicability of these microbial fuel systems to scale up the electricity generation. Under ideal laboratory conditions these system produced power of only 2-20 mW (Ren *et al.* 2007). However, the amount of biomass-based energy in microbial processes is very low. It has yet to reach to its full potential to work in real field pilot scale power production.

Chhattisgarh is an emerging agro-industrial state. The state produced tons of seasonal crops. In the year 2012 and 2013, production of 8,127.5 and 1,885.67 thousand metric tons of Kharif and Rabi crop was reported (Chhattisgarh Economic Census, 2013-14). This agro-industrial profile of the state supports many large and medium as well as small size industries. Thus, the waste generated from this region is rich in biodegradable materials. Also, easy availability of the industrial wastes offers the opportunity to work in this concerned field.

Brief review of the work already done in the field

Energy is the inseparable part of the human life which is the indicator of the growth and development of human society. Over a last few years the attraction for sustainable energy production via waste has intensified the research in the field of microbial fuel cells. The concept of electro-physiological phenomenon came into existence in the year 1911 when Potter studied chemical changes associated with electrical change. This preliminary investigation utilized *Saccharomyces* and common nutrient glucose as substrate. His work was enough to open up a large field of work on electrical microbial fuel (EMF) system. In 1982, Wingard *et al.* defined the mechanism of the electrochemical reactions in the MFC and developed methodology. In 1984, Delaney *et al.* tested effectiveness of redox mediators in microbial fuel cells containing *Alcaligenes eutrophus, Bacillus subtilis, Escherichia coli* and *Proteus vulgaris* as the active biological agent.

Kim *et al.* (2000) tested thionin as a mediator to evaluate charging-discharging characteristics of the MFC using *Proteus vulgaris*. Park *et al.* (2001) suggested a novel strain of the genus *Clostridium,* C. *butyricum* with electrochemical and Fe(III) reducing activities as an electron sink in mediator-less microbial fuel cell using starch processing wastewater. Kim *et al.* (2002) studied different *Shewanella putrefaciens* strains in a mediator-less MFC. The first report of microbial electricity production solely by cells attached to an electrode was discovered using *Geobacter sulfurreducens* by Bond and Lovley (2003). Gil *et al.* (2003) emphasised on various factors for optimization of operating conditions such as pH, resistance, electrolyte used, and dissolved oxygen concentration for current generation.

Liu and Logan (2004) examined bacterial isolates from domestic wastewater as biocatalyst and glucose as substrates for power generation in an air-cathode MFC. Gregory *et al*. (2004) reported *Geobacter metallireducens* in sediment based system demonstrating that electrodes may serve as a direct electron donor for anaerobic respiration. Their finding implicated for the harvesting of electricity from anaerobic sediments and for the bioremediation of oxidized contaminants.

Kim *et al*. (2005) studied two-chambered MFC as film bioreactor for wastewater treatment using anaerobic sewage sludge. Bond and Lovley (2005) worked on MFC with oxygen as the electron acceptor. They used *Geothrix fermentans* to utilize lactate, malate,

propionate, and succinate *etc.* as substrate. This was the first report on electrode reduction from the complete oxidation of an organic compound by an isolate. Oh and Logan (2005) studied glucose fermentation in food processing wastewater and simultaneous production of hydrogen and electricity. Lovley (2006) explained the electricigens like *Geobacter* and *Rhodoferax* to go insights into the elegance of extracellular electron transfer. His work developed the better understanding toward application of these electricigens. Reimers *et al.* (2006) reported benthic microbial fuel cells coupled biogeochemical reactions monitored for 125 days and recorded a maximal sustained power density of 34mW/m^2 that was equivalent to 1100 mW/m^2 of the sea floor.

Ringeisen *et al.* (2007) studied mini-MFC using an aerobic culture of *Shewanella oneidensis*. They found maximum power of 0.40 mW and 0.6 mW in aerobic and anaerobic conditions, respectively. Richter *et al.* (2007) reported current production using a co-culture of *Pelobacter carbinolicus* and *Geobacter sulfurreducens* with ethanol as the fuel suggesting the mechanisms for extracellular electron transfer to Fe (III) oxides and fuel cell anodes. Jung and Regan (2007) studied characterizations of MFC anode biofilms that contained *Geobacter sulfurreducens* and an uncultured bacterium clone. Huang and Logan (2008) examined treatment of a paper recycling wastewater with a power density of 144±7 mW/m^2 using an unamended wastewater. Min *et al.* (2008) studied the effect of different temperature on bioelectricity production and found maximum power density of 43 mW/m^2 at 22^0C. Wang *et al.* (2009) discovered the effectiveness of close electrode spacing on power generation using flat carbon cloth and carbon paper materials electrodes in an MFC.

A comparison of power densities and oxidation–reduction potentials between *Shewanella oneidensis* MR-1 and mixed cultures from wastewater inoculum was performed by Watson and Logan (2010). The modification in MFC architecture takes place with the advancement in techniques. A prototype up-flow single- chambered microbial fuel cell (MFC) with brewery wastewater was studied by Katuri and Scott (2010). The variation in substrate and MFC configuration is reported these years. Goud and Mohan (2011) worked on pre-fermented food waste and compared the current production with non-fermented wastes. Pentachlorophenol was used for power generation by Huang *et al.* (2012). A bi-function MFC for wastewater treatment and power generation was examined by Liu *et al.* (2013). Cheng *et al.* (2014) proposed a simple model of

leading-out terminals to stimulate more power production. Wang *et al*. (2014) developed tubular membrane MFC reactor for COD real-time monitoring and bioelectricity generation simultaneously. Recently, the researchers emphasizes on the scale up and more power production.

The present research work is designed to study following objectives:

1. Collection of substrates from rice based industries (rice bran oil extraction industry and rice processing industry).
2. Designing and construction of the microbial fuel cell and comparative study of different electrode materials.
3. Physiochemical analysis of substrate before and after operation.
4. Isolation and screening of electrogenic bacteria.
5. Effect of different parameters on bioelectricity production.
   i. Effect of buffer strength on bioelectricity generation.
   ii. Effect of different external mediator.
   iii. Effect of concentration of substrate.
   iv. Effect of addition of external nutrients.
6. Comparative study of electrogenic property of isolated identified bacterial strain with known electrogenic bacterial strain.
7. Analysis and interpretation of data.

Noteworthy contribution in the field of proposed work

Energy is gaining more importance now-a-days. Rapid depletion of non-renewable resources stimulated human to think for alternative renewable resources. The microbial fuel cells are a step towards the solution of these problems. The current scenario of the concern field will be discussed in this section. Liu *et al.* (2004) demonstrated the production of bioelectricity (26 mW/m^2) in a MFC while removing up to 80% of the COD from domestic wastewater. Fermentation technology was coupled with microbial fuel cell technologies to produce hydrogen and electricity from a food processing wastewater by Oh and Logan (2005). They reported a maximum power density of 81 ±7 and 371 ±10 mW/m^2 in a dual and single chambered MFC, respectively.

Aelterman *et al.* (2006) studied industrial organic influent and effluent streams from a potato processing factory and found highest power output of 58±2.1 W/m^3 from the influent of the anaerobic digester. Feng *et al.* (2008) examined effect of temperature on brewery wastewater and recorded that, decrease in temperature from 30°C to 20°C reduced the maximum power density from 205 mW/m^2 to 170 mW/m^2 also when buffer strength increased from 50 mM to 200 mM the power density increases upto 528 mW/m^2. Lu *et al.* (2009) examined starch processing wastewater in an air-cathode MFC and obtained maximum voltage output and power density of 490.8 mV and 239.4 mW/m^2 and current density of 893.3 mA/m^2.

Patil *et al.* (2009) carried out investigation on chocolate industry wastewater with activated sludge inoculums in two-chambered microbial fuel cell. They recorded maximum current of 3.02 and 2.3 A/m^2 in glucose-fed MFCs with membrane and salt bridge, respectively. A model of single air-cathode microbial fuel cell using continuous beer brewery wastewater for electricity production was proposed by Wen *et al.* (2009). They reported an open-circuit voltage of 0.578 V and a maximum power density of 264 mW/m^2. A maximum volumetric power production (1.10 W/m^3; 308 mV; 1.78 mA) was observed while studying real field dairy wastewater by Mohan *et al.* (2010). Behera *et al.* (2010) evaluated the performance of earthen pot MFC and a proton exchange membrane MFCs while treating rice mill wastewater and found maximum sustainable volumetric power of 2.3 W/m^3 and 0.53 W/m^3, respectively.

Wen *et al*. (2010) provided a new approach for brewery wastewater treatment in conjugation with electricity generation in double-chamber microbial fuel cell (MFC) having carbon fiber as anode and plain carbon felt with biofilm as cathode. Patil *et al*. (2011) demonstrated a comparative study on sugar wastewater, dairy wastewater and paper wastewater by using *Enterobacter aerogenes* NCIM 2340 with mediator neutral red and toludine blue and got highest power density of 16.347 mW/m^2, 34.274 mW/m^2 and 42.646 mW/m^2, respectively. Kalathil *et al.* (2012) developed a novel single chamber biocathode- MFC without using any expensive materials for the simultaneous decolourization of real dye wastewater and electricity generation. They recorded power density of 8 W/m^3 indicating that GAC-biocathode can be a good alternative to other chemical catalysts. Mohanakrishna *et al*. (2012) investigated effluents from a pulp and paper mill using carbon nanotubes and carbon nanopowder as electrodes generating highest current densities up to 6 A/m^2.

Elakkiya and Matheswaran (2013) reported highest power density of 192 mW/m^2 and volumetric power of 3.2 W/m^3 with aerobic MFC and power density of 161 mW/m^2 and volumetric power of 2.7 W/m^3 with anaerobic MFC using dairy industry wastewater. Liu *et al*. (2013) utilized domestic wastewater for the electricity production. They succeeded in producing maximum power of 21 mW/m^2 and a current of 0.86 mA from the MFC without a proton exchange membrane. Baranitharan *et al.* (2013) gone through the examination of double chamber microbial fuel cell fed with Palm oil mill effluent for power generation recovering the maximum power density and volumetric power density of 45 mW/m^2 and 304 mW/m^3, respectively. Majumder *et al*. (2014) used wild-type *Pseudomonas putida* for refinery wastewater treatment and bioelectricity generation in an air-cathode chamber over four-batch cycle. They found maximum voltage of 355 mV and power density of 0.005 mW/cm^2 in the third cycle.

Thus, the proposed work is a step towards finding a new way to utilize effluents of rice based industries in microbial fuel cell using electrogenic bacteria, so that maximum amount of electricity can be produced to fulfil the future requirements.

Proposed Methodology

- **Collection of wastewater sample and physiochemical analysis**

  - The effluent will be collected from the rice based industries (rice bran oil extraction industry and rice processing industry) containing rich starchy material. Samples will be collected quarterly in sterile flasks and will be stored at 4±1 ^{0}C.

  - The substrate will be analysed before and after the operation for different physiochemical parameters- colour, odour, pH, BOD, COD and total suspended materials using standard methods.

- **Microbial fuel cell configuration and optimization**

Construction

- H-shaped microbial fuel cells will be architect.
- Each chamber will be made up of autoclavable plastic material.
- The anodic and cathodic chamber will be connected using an agarose salt bridge.
- Comparative estimation of different electrodes like graphite, carbon, Zn, Cu and Al will be performed.

Operation

- Sterilization of MFC assembly will be done.
- Anodic chamber will be filled with substrate and cathodic chamber will be an open air cathode chamber containing phosphate buffer.
- Optimum conditions- temperature and pH will be maintained.

- **Isolation and characterization of screened electrogenic bacteria (morphological, biochemical and molecular)**

Isolation

- Isolation of bacterial strain will be done on nutrient agar medium plates at optimum temperature will be incubated for 24-48 hr.

Screening

- Isolates will be screened for their electrogenic property and pure culture will be maintained.

Characterization

- Morphological studies will be done using gram's staining and other staining methods.
- Biochemical test (IMViC test, amylase production test, fermentative test) will be done.

Identification

- Molecular identification of maximum bioelectricity producing bacteria will be done.

➢ **Effect of different parameters on bioelectricity generation**

1. Effect of buffer strength on bioelectricity generation: - phosphate buffer of different concentration will be checked for the optimum bioelectricity generation.

2. Effect of different external mediator: - mediators are known to enhance the mobility of electrons, so it is important to check the ability of different mediator for production of bioelectricity.

3. Effect of concentration of substrate: - different concentration of substrate will have different organic load which may affect the bioelectricity generation.

4. Effect of addition of external nutrients: - nutrients play very important role in growth of bacteria so the effect of additional nutrients will be checked.

➢ **Analysis and interpretation of data**

- Analysis of recorded data using required standard methods, research methodological tools and software.

Expected outcomes of the proposed work

21st century is pretty much concerned about bioenergy, based on clean and green technologies. Use of conventional non-renewable resources causes high capital loss. To overcome this problem a high strength wastewater from rice based industries offers a better alternative. The expected outcome of the proposed work is to utilize wastewater from rice based industries and to reveal the potent electrogenic bacteria which are helpful in bioelectricity generation. Microbial fuel cells are emerging as a simple and sustainable technology that will contribute to the dual function of bioelectricity generation and wastewater treatment.

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